

Tests of Hypotheses on the Adaptive Value of an Extended Male Phase in the Hermaphroditic Shrimp *Lysemata wurdemanni* (Caridea: Hippolytidae)

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Abstract. Hypotheses on delayed sex change in the protandric simultaneous hermaphrodite *Lysemata wurdemanni* were tested with observations from population samples, mating experiments, and experiments on sex change under optimal and suboptimal breeding conditions. Male-phase individuals (MPs) much larger than the minimum size of sex change were most frequent in a natural population from fall through early spring. The hypothesis was tested that some MPs delay sex change to the simultaneous hermaphrodite female-phase (FP) because MPs are more competitive in obtaining copulations with parturial FPs than are FPs mating as males (MFPs). In different experiments, parturial FPs were maintained with two potential male mating partners (large MP and MFP, small MP and MFP, large MP and small MP) through the parturial molt and spawning; activities were recorded with time-lapse video. MFPs gained the single copulation with the parturial FP as frequently as MPs, large or small, but large MPs copulated with more FPs than small MPs. The hypothesis of FP reversion to large MP was tested experimentally and rejected. Rate of change of MP to FP was much lower in large MPs maintained under suboptimal (fall/winter) than optimal (spring/summer) breeding conditions. The results presented here suggest that the occurrence of large MPs from the fall to early spring is better explained by abiotic proximate factors related to breeding than by socially mediated sex change in different demographic environments.

Introduction

Many organisms, both plant and animal, partition reproductive output into male and female components. Such sex allocation may occur before fertilization or during embryonic development. In hymenopteran insects (ants, wasps, bees), haplodiploid sex determination allows females to produce male and female offspring in variable proportions (Wilson, 1976; Thornhill and Alcock, 1983). In turtles and some other reptiles such as alligators, sex determination is temperature dependent, so that females may control the sex ratio of offspring by the seasonal timing of egg laying or the location of nests (Bull, 1980; Ferguson and Joanen, 1982). Higher male fetal mortality may occur in mammalian females under stressful conditions, resulting in female-biased litters (Wilson, 1976; see also Charnov, 1982). In other organisms, sex allocation may occur after birth or hatching in the sexually mature organism. Simultaneous hermaphrodites may vary resources to male and female gamete production (Charnov, 1982). In sequential hermaphrodites, sexual function is partitioned ontogenetically because an individual first reproduces as one sex and then the other (sex changers; Ghiselin, 1969; Warner, 1975; Charnov, 1982; Policansky, 1982). Simultaneous and sequential hermaphroditism is found in many plant taxa, in most invertebrate groups (except insects), and in many fishes (Chan and Yeung, 1983; Ross, 1990). The direction of sex change (female to male; protogyny; male to female; protandry) is fairly well explained by the size-advantage model (Ghiselin, 1969), especially in fishes and crustaceans, two groups in which sequential hermaphroditism is relatively common (Warner, 1975). What is not often well explained, especially in decapod shrimps, is variation in the timing of sex change, *i.e.*, the amount of time spent as male and female.

Protandric hermaphroditism occurs in several genera and

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Abbreviations: AG, androgenic gland; CL, carapace length; ESD, environmental sex determination; FP, female-phase individual (simultaneous hermaphrodite in *Lysemata wurdemanni*); MP, male-phase individual; MFP, a FP acting as a male mating partner; PFP, parturial FP; PSH, protandric simultaneous hermaphroditism; TR, transitional individual.

families of caridean shrimps (Bauer, 2000). In the simplest form of caridean protandry, all individuals develop first as males and then later change to breeding females. The sexual system may be more complex, with a mixture in the population of primary females, which develop only as females, and protandric individuals, as in *Pandalus jordani*, *P. borealis*, *P. eous*, *P. montagui*, and *P. danae* (Butler, 1980; Bergström, 2000). In other species, the population may consist of protandric individuals and primary males, individuals that never change to females, as in *Thor manningi* (Bauer, 1986) and *Athanas kominatoensis* (Nakashima, 1987).

Throughout the last century, *Lysmata seticaudata* was described as a completely protandric species (Spitschakoff, 1912; Dohrn, 1950; Charniaux-Cotton and Payen, 1985). Smaller individuals have external male characteristics, but the gonads are ovotestes with immature oocytes in the ovarian region. With increasing size, individuals lose external male characters and change to breeding females that incubate embryos. Unlike other protandric carideans, the female-phase individuals (FPs) retain the male ducts and testicular portion of the ovotestes, and sperm production continues (Berreur-Bonnenfant and Charniaux-Cotton, 1965; Charniaux-Cotton, 1975). The ability of *L. seticaudata* FPs to function as males was not addressed in these studies. However, FPs of *Lysmata wurdemanni* (Bauer and Holt, 1998) and *L. amboinensis* (Fiedler, 1998), with sexual changes during ontogeny similar to *L. seticaudata*, mate as males and fertilize other FPs, although they are unable to fertilize themselves. They are functional, outcrossing simultaneous hermaphrodites. Bauer (2000) termed this sexual system "protandric simultaneous hermaphroditism" (PSH), in which male-phase individuals (MPs) function only as males, but FPs are functional simultaneous hermaphrodites. Morphological and behavioral observations on these and other *Lysmata* spp. indicate that PSH is widespread in the genus (Bauer, 2000, 2001).

The size (age) of sex change in protandric species may be variable (Charnov *et al.*, 1978; Charnov, 1981; Charnov and Anderson, 1989; Bergström, 1997, 2000). The size of sex change may be a phenotypically flexible trait governed by the demographic environment in which an individual occurs (environmental sex determination, or ESD; Charnov *et al.*, 1978; Charnov, 1981; Charnov and Anderson, 1989). In *Pandalus* spp. for example, a male in a population with abundant females might gain more inseminations by delaying change to female because males can potentially inseminate several females during the breeding season. A *Pandalus* female, in contrast, produces only one brood per year. When females are not abundant in the population, a male might benefit by early change to female because insemination of a female's brood of eggs is assured. As a male, the individual would have to compete with numerous other males to inseminate one of the scarcer females. On the other

hand, a male changing to female later in life would begin female production with larger broods, since female fecundity is correlated with size in carideans including *Pandalus* spp. (Bauer, 1991; Bergström, 2000). However, a later change to female might cause the loss of female breeding for an entire season, given only one brood per year, thus countering the fecundity advantage of late change to female. The relative proportions of males and females in the population, perceived by an individual through social interactions, would serve as the environmental factor stimulating or inhibiting male change to female.

However, the hypothesis of ESD in *Pandalus danae* was rejected by Marliave *et al.* (1993) after they conducted extensive experiments involving the rearing of a large number of young males in different demographic environments. They concluded that the sexual composition of the population has no effect on sex determination (change) in this protandric species. Other mating and rearing experiments led Marliave *et al.* (1993) to conclude that the timing of sex change was under genetic control. They proposed that any adaptive change in the proportions of sexual morphs would occur by between-generation selection on sexual genotypes rather than a within-generation, socially determined response as proposed by Charnov *et al.* (1978) and Charnov (1981). Bergström (1997), on the basis of results of a demographic analysis, concluded that the variable type and size of sex change in *Pandalus borealis* was best explained by frequency-dependent selection on genotypes for sex change at different ages (sizes). According to this hypothesis, different combinations of mortality, recruitment, and growth favor different sex-change genotypes. Conover and Munch (2002) showed experimentally in the fish *Menidia menidia* (Atherinidae) that size-dependent selection, like that acting on commercially fished populations, lowers the age of sexual maturity. Charnov *et al.* (1978) and Charnov (1981) reported a decrease in the size (age) of sex change in fished pandalids. This might result from size-related mortality (selection) (Conover and Munch, 2002) rather than from an adaptive response (decision) by individuals to demographic changes in the population. On the other hand, Zupo (2001) clearly showed that seasonal variations in diet of newly recruited shrimps determine whether or not sex change takes place in the protandric shrimp *Hippolyte inermis*.

In *Lysmata wurdemanni*, individuals from a natural population vary in size from 3–14 mm carapace length (CL) (Bauer and Holt, 1998; Bauer, 2002). Individuals enter the population as MPs and can change to FPs at a size as small as 6 mm CL; the majority change by 7.5 mm CL. However, there is frequently considerable overlap between MP and FP size-frequency distributions. Many MPs do not change sex until a much larger size, with some MPs becoming as large as the larger FPs (Bauer and Holt, 1998; Bauer, 2002). Some MPs might never change to FP, although all are

morphologically capable of doing so (Bauer and Holt, 1998; Bauer, 2000; Lin and Zhang, 2001). Bauer and Holt (1998) and Bauer (2000, 2001) suggested that delayed change to the reproductively advantageous FP, a simultaneous hermaphrodite, might be selected for in certain demographic situations if large MPs were competitively superior to FPs (mating as males) and small MPs in inseminating FPs near spawning. Lin and Zhang (2001) reared MPs of *L. wurdemanni* singly and in groups of from 2 to 10 individuals, finding that change to FP increased with increasing MP group size. Replicate size was quite small in this experiment, varying from 2 to 9 replicates per treatment. The authors concluded that these results demonstrated social control of sex ratio. Furthermore, they proposed that persistence of MPs in some treatments suggested a mating advantage for large MPs in certain demographic situations.

Other explanations are possible for large MP size in *Lysmata wurdemanni*. Large MPs might be FPs that have reverted to the male phase. Bidirectional sex change is known in some animals, such as gobiid fishes (Munday, 2002) and polychaetes (Berglund, 1986). A secondary change back to male purportedly takes place in some females of the protandric alpheid *Athanas indicus* (Suzuki, 1970). In *L. wurdemanni*, abiotic factors related to female breeding might account for the seasonally related occurrence of large MPs. The largest MPs, including transitional MPs changing to FP, are found in winter and early spring (Bauer, 2002). Suboptimal conditions for breeding (lower water temperatures, short daylength) and presumably larval survival (planktonic food supply) occur during the late fall through early spring in this primarily tropical species studied near the northern edge of its range (Bauer and Holt, 1998; Bauer, 2002). Abiotic environmental conditions unfavorable for female breeding might cause a decrease in female hormone levels, resulting in a shift in female/male hormones in favor of the male system. Female-phase individuals, with both male and female reproductive systems, might molt back into a male phase, with male external sex characters. Alternatively, suboptimal breeding conditions might delay change to FP in MPs that reach the size of sex change during the fall, resulting in growth to the very large MPs observed by Bauer (2002) in the late winter and early spring.

The objective of this study was to examine components of hypotheses explaining the presence of large MPs in *Lysmata wurdemanni*. The male mating abilities of large MPs, small MPs, and FPs were compared to determine the possible male mating advantages of delayed sex change. The presence of large MPs might alternatively be explained by factors not related to the sexual composition of the population and socially determined sex change. The possible effects of seasonal abiotic factors unfavorable to female breeding resulting in either (a) reversion of FPs to large MPs or (b) delayed MP change to FPs were examined by

maintaining (a) FPs and (b) MPs capable of sex change under suboptimal (nonbreeding season) and optimal (breeding season) conditions.

Materials and Methods

Population samples and live specimens were collected from a population of *Lysmata wurdemanni* inhabiting the rock jetty at Mustang Island, Port Aransas, Texas (27° 50' N, 97° 03' W). Collection was during low tides at night (Bauer, 2002): when tides are lower than -0.5 ft mean sea level, the rock ledge and tidepool habitats of these shrimps are exposed, and the shrimps are active at night, moving out into pools and the perimeter of the jetty rocks where they can be taken with dipnets. Hand dipnets were used to take the shrimps from tidepools; long-handled dipnets were more effective in sweeping deep under rock ledges. All nets had a 1-mm mesh and were effective in collecting the smallest (= newly recruited) individuals of the population (Bauer, 2002). In any particular location, groups of individuals showed no apparent segregation by sexual morph or size, and the samples are assumed to represent a random sample of the population. Population samples were taken monthly from June 1999 through June 2000, and then bimonthly from August 2000 through June 2001 (Bauer, 2002). Shrimps collected in population samples were first preserved in 10% seawater formalin and later transferred to 70% ethanol for permanent storage. Live specimens for experiments were collected separately from population samples and transported in sealed collecting bags containing seawater oxygenated just before the 8–10-h trip from the collecting site to the home institution.

Sexual morphs were identified and classified as in Bauer and Holt (1998). Male-phase individuals (MPs) have cincinnuli (coupling hooks) on pleopod 1 endopods and appendices masculinae on pleopod 2 endopods. Transitionals (TRs) are MPs in which the ovarian portion of the ovotestes is filling with vitellogenic oocytes, which is easily observed through the carapace in living and preserved specimens. At the next molt, TRs change to FPs and spawn for the first time. Female-phase individuals (FPs) lack pleopod 1 cincinnuli and appendices masculinae. They are typical caridean females in external morphology, and they brood embryos. However, they can mate as males (simultaneous hermaphrodites) (Bauer and Holt, 1998). As in females of other carideans, FPs of *L. wurdemanni* near spawning (ovarian portion of gonad full of vitellogenic oocytes) become receptive to mating immediately after a prespawning (parturial) molt (Bauer and Holt, 1998; Bauer and Abdalla, 2001). As in other carideans, the male deposits an external spermatophore on the underside of the female, and there is no sperm storage (Bauer, 1976; Bauer and Holt, 1998). In this report, FPs very near or just completing the parturial molt are termed parturial FPs (PFPs).

Mating experiments tested the hypothesis of no difference in male mating ability among FPs (mating as males = MFPs), small MPs, and large MPs. In three experiments, competition for inseminating the PFP was between a large MP and a MFP, a small MP and a MFP, and a large MP and a small MP. Parturial FPs near the prespawning molt were identified by gonad condition (vitellogenic oocytes filling the cephalothoracic space behind the eyes and above the cardiac stomach) and developmental state of brooded embryos (near hatching, with large eyes and little or no yolk). MFPs carried embryos but, unlike PFPs, were not close to a parturial molt. Small and large MPs were defined as MPs ≤ 6.5 mm CL and > 6.5 mm CL, respectively. The size of 6.5 mm CL was chosen to define "large" MP because MPs can change to FPs above this size (Bauer and Holt, 1998; Bauer, 2002).

In each replicate, a premolt PFP was placed with two potential mating partners. The median duration of replicates, from placement of individuals into the experimental chamber until spawning by the PFP, was 2 days (1–9 d, $n = 90$). The MP or MFP that copulated with and inseminated the PFP was identified from time-lapse video recordings. Replicates in which the PFP copulated with more than one male mating partner (4 of 94 replicates) were not included in the data analyses. In all other replicates, there was only a single copulation, except for one replicate in which a MFP copulated twice with the PFP. Spawned PFPs were maintained alive for 5 days to determine if the attached brood of eggs had been fertilized and contained developing embryos (Bauer and Holt, 1998).

Replicates of mating experiments were conducted in aquaria partitioned into a space 20 cm wide \times 25 cm long \times 15 cm high to allow for sufficient magnification using the CCD surveillance video camera, which was equipped with an 8-mm lens sensitive to both low-intensity white and infrared light. Activities were recorded with a time-lapse video at a speed of 5 pictures per second (24-h recording mode). Fluorescent and infrared (880 nm) lamps provided day and night illumination, respectively. In replicates with a large and a small MP, individuals had to be marked to distinguish between them in videotapes, especially at night when most mating occurred. Small pieces of reflective tape were fixed with cyanoacrylate glue on the carapace of one MP and the posterior abdomen of the other, with position assigned at random. No marking was needed in replicates with a MP and a MFP because the latter could be identified by its brood of embryos. After hatching of embryos prior to the mating molt, the PFP was identified by the absence of embryos and the presence of vitellogenic ovotestes. Reflective tape, embryo broods, and vitellogenic gonads show clearly on videotapes recorded with infrared (nighttime) illumination. As in Bauer and Holt (1998), shrimps were maintained prior to and during experiments at water temperatures of 25–28 °C, salinities of 33–36 ppt, and a pho-

toperiod of 14-h day: 10-h night. Shrimps were fed shrimp pellets daily.

The hypothesis that FPs may revert to large MPs was tested by an experiment in which FPs were maintained under favorable and less favorable breeding conditions for 9 weeks. In the "optimal" treatment (2 September through 2 November, 2000), 50 FPs with embryos were maintained in groups of 10 individuals occupying 38-l aquaria at a water temperature, controlled by aquarium heaters, of 26 °C and a photoperiod of 14-h light: 10-h dark. In the "suboptimal" treatment (22 September to 24 November, 2000), another 50 FPs with embryos were similarly maintained except without heaters so that water temperatures would be cooler than those in the optimal treatment. The ambient temperature of the aquatic laboratory during this period was 19 °C; the photoperiod was set at 10-h light: 14-h dark. Prior to the experiment, individuals used in both treatments were maintained at a salinity of 33–36 ppt and an optimal temperature (26 °C) and photoperiod (14-h light: 10-h dark). They were fed daily, and the aquarium water was partially changed weekly. To reduce cannibalism of newly molted shrimps, which was an observed source of mortality during this experiment, small concrete block shelters were placed in each aquarium for this thigmotactic species. Molts (exuviae) readily visible in the aquaria were removed and recorded daily. Absolute counts of molts were not feasible because (a) the shrimps may pick apart and eat some exuviae before they are observed, and (b) other exuviae are hidden by shelters and aquarium tubing and could not be observed without major disturbance of the aquarium environment. At the end of the experiment, all individuals were preserved and checked for sexual condition.

The hypothesis that sex change of large MPs to FPs is greater under optimal (spring/summer) than suboptimal (fall/winter) breeding conditions was tested with an experiment using a setup similar to that just described for the previous experiment. The MPs used in both treatments (all nontransitional) were maintained at optimal temperature and photoperiod prior to the beginning of the experiment. In the optimal treatment ($n = 46$ MPs), water temperature was maintained with heaters at 26 °C, and photoperiod was 14-h light: 10-h dark. In the suboptimal treatment ($n = 45$ MPs), cooler water temperatures were obtained by not using water heaters, with water temperature following the ambient air temperature of the laboratory, which was not under control of the investigator. The experiment was begun when a decrease in ambient laboratory temperature was predicted from outside weather conditions. Water temperature in the suboptimal treatment was 23 °C at the beginning of the experiment and decreased to 18–19 °C (weekly median temperature = 20 °C); photoperiod was 10-h light: 14-h dark. After 14 weeks (20 October 2001 to 23 January 2002), the laboratory air temperature and thus the water temperature of the suboptimal treatment began to rise. Heaters were

then placed in aquaria of the suboptimal treatment to increase water temperature to that of the optimal treatment, and photoperiod was changed to 14-h light: 10-h dark, simulating a change from fall/winter to spring/summer conditions. The experiment then continued for another 8 weeks. The shrimps were maintained and checked for molts as in the previous experiment. Male-phase individuals that changed to FP (brood of embryos observed) were removed from the experiment and preserved. When only one MP remained in an aquarium, it was removed when it reached the late transitional stage (mature vitellogenic ovotestes), with the assumption that it would have transformed to FP at the next molt. This was done because it could not have produced a brood of embryos (visible evidence of sex change) upon molting to FP without a mating partner.

Results

Occurrence of large MPs

Large MPs, defined as MPs > 6.5 mm CL, composed 17.6% to 100% of the MP population (Fig. 1), with higher proportions of large MPs in the fall through early spring

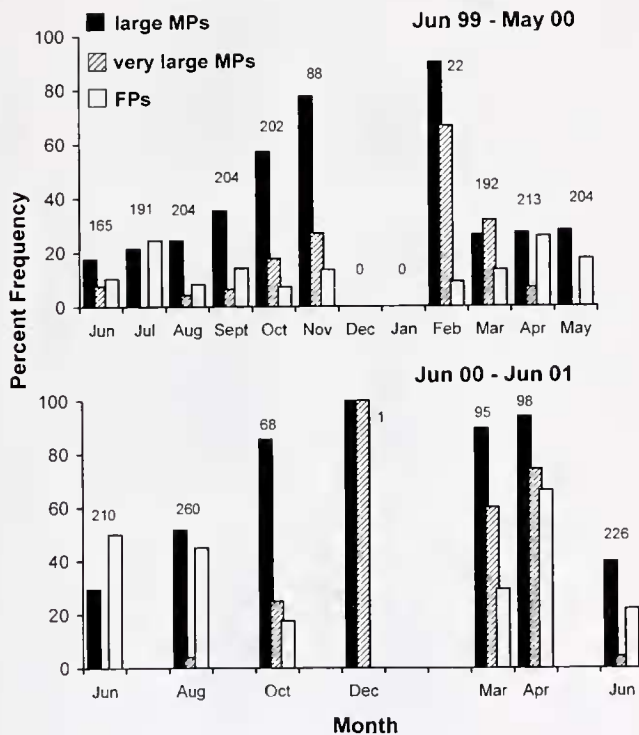


Figure 1. Temporal variation in percent frequency of large and very large male-phase (MP) individuals, and of female-phase (FP) individuals. Frequency (converted to percent) for large MPs = (# large MPs)/(# total MPs); very large MPs = (# very large MPs)/(# large MPs); FPs = (# FPs)/(total # individuals in population sample). Percent total MPs = 100 - % FPs. Months in which sampling occurred are listed on the abscissa. The number of individuals in population samples is given above the histogram bars for each month.

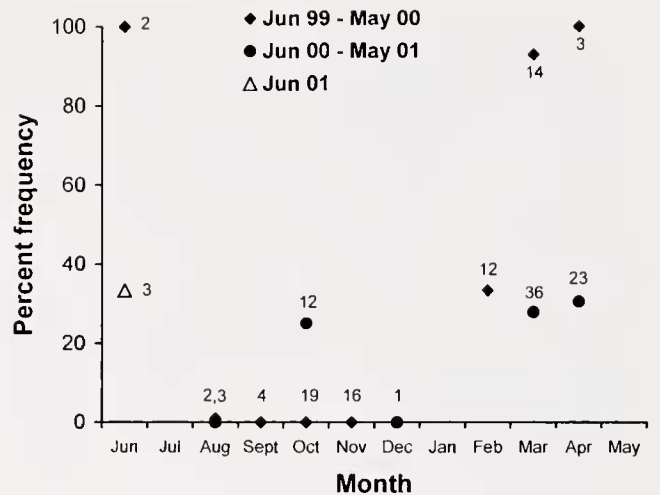


Figure 2. Temporal variation in percent frequency of very large MPs in the transitional stage (TR) to female-phase. The number with each data point is the sample size of large MPs on which frequencies were calculated.

(Fig. 1). The proportions of large MPs ≥ 8.5 mm CL (“very large MPs”) followed a similar pattern. Some (28.7%; n = 150) of the very large MPs were transitionals that would change to the female phase at the next molt. The highest frequency of these very large transitionals occurred from mid-winter through late spring (Fig. 2).

Mating experiments

Precopulatory and copulatory behaviors were qualitatively similar to those described in Bauer and Holt (1998), in which a single FP served as the male mating partner, except for the following observations. In this study, MPs and MFPs began to approach, follow, and remain near the PFP as the latter neared the parturial molt. These behaviors became obvious about 1 h before the PFP molt. In addition, another behavior was observed (“perching”) in about 20% of the replicates, in which a MP or a MFP perched on the PFP from above and remained in that position as long as several seconds. Although individuals of *Lysmata wurdemanni* can be aggressive—for example, fighting over food particles using the third maxillipeds and anterior pereopods—no obvious aggressive behavior between the potential male mating partners over a parturial female was observed in the videotapes.

The hypothesis of no difference among sexual morphs in their ability to copulate with and inseminate parturial females (PFPs) was tested with mating experiments. In the experiment with large MPs and small MPs as potential male mating partners (Table 1), large MPs gained the single PFP copulation in more replicates than small MPs (Fig. 3A). The hypothesis of no difference from a 50:50 distribution of copulations between large MPs and small MPs was tested with chi-square and is rejected ($\chi^2_1 = 6.53$; $P = 0.01$; $n =$

Table 1

Size data for potential male mating partners placed with newly molted parturial females in mating experiments

Experiment	Potential Male Mating Partners		
	SMP	LMP	MFP
SMP vs. LMP	5.4 (4.4, 6.4)	7.4 (6.6, 9.4)	
LMP vs. MFP		7.7 (6.6, 9.7)	8.2 (6.9, 10.0)
SMP vs. MFP	5.6 (4.5, 6.5)		8.8 (7.2, 12.3)

Size given is median carapace length (mm), with minimum and maximum values in parentheses ($n = 30$ for all categories). LMP, large male-phase; MFP, non-parturial female-phase (simultaneous hermaphrodite); SMP, small male-phase.

30). In another experiment, large MPs and MFPs (Table 1) were equally successful in obtaining the PFP copulation (Fig. 3B) ($\chi^2_1 = 0$; $P = 1$; $n = 30$). In the 19 replicates in which the large MP was smaller than the MFP, the MP and MFP gained the PFP copulation equally ($n = 9, 10$ respectively). Similarly, when the large MP was larger than the MFP ($n = 8$ replicates), each gained the single PFP copulation in an equal number of replicates. In the small MP vs. MFP experiment (Table 1), MFPs obtained the PFP copulation in more replicates (Fig. 3C), but the difference is not statistically significant ($\chi^2_1 = 1.2$; $P = 0.27$; $n = 30$). The copulated PFP produced a fertile brood of embryos in 87 of the 90 replicates of the three experiments.

Correlations of large MP and FP relative abundance

The effect of demographic influence (relative abundance of FPs) on the proportion of large MPs was investigated. From population samples, Spearman rank correlation coefficients (r_s) were calculated for possible correlations between % large MP and % FP, and % very large MP and % FP (Fig. 1). The null hypothesis of no correlation ($r_s = 0$) between % large MP and % FP and between very large MP and % FP was accepted ($r_s = 0.02$, $P \gg 0.20$ and $r_s = -0.32$, $P > 0.20$, respectively; $n = 17$ months in both tests).

FP to MP sex change experiment

This experiment tested the hypothesis that FPs may change back to large MPs. In the treatment with optimal breeding conditions, all surviving individuals (36 of 50) were FPs. Of these 36 FPs, 33 (91.6%) showed female reproductive activity, either incubating embryos (21) or, if without embryos, showing mature vitellogenic ovaries (12), indicating spawning at their next molt. In the suboptimal treatment, the 39 survivors out of 50 were all FPs. Of these 39, 33 (84%) were reproductively active as females; 26 incubated embryos, and 7 without embryos had mature

vitellogenic ovaries. During the 9 weeks of the experiment, 93 exuviae were collected in the optimal treatment vs. 43 in the suboptimal treatment. The molting rate, adjusted for number of survivors per treatment, was 2.4 times greater in the optimal treatment.

Large MP to FP sex change experiment

During first 14 weeks, 42 of 46 individuals in the optimal treatment changed to FP (including 2 solitary late-stage transitionals), 2 died, and 2 remained MP; in the suboptimal treatment only 23 of 45 changed (5 deaths). The rate of change was relatively constant in the optimal treatment compared to that of the suboptimal treatment (Fig. 4A), in which the rate of sex change slowed with decreasing ambient temperatures (Fig. 4C). Molting rates were always higher in the optimal treatment (Fig. 4B). The mean frequency of individuals molting per week was 31.2% in the optimal and 13.0% in the suboptimal treatment. Given that the period between molts may be estimated by the inverse of

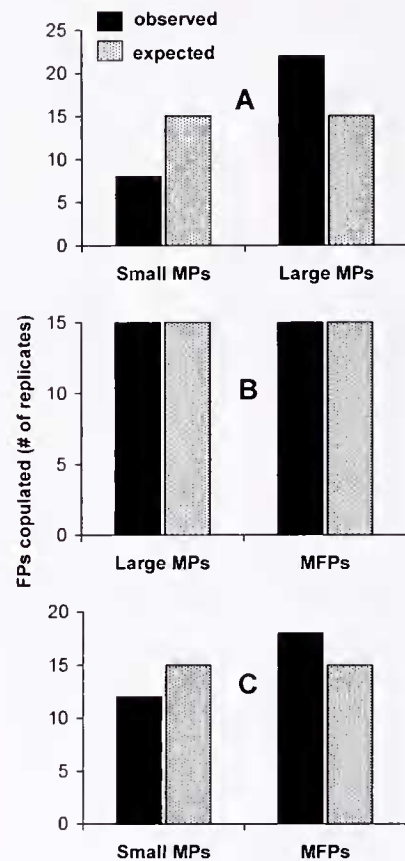


Figure 3. Comparative mating success of sexual morphs. The observed number of replicates (black fill) in which one of the other of two sexual morphs obtained the single copulation with the parturial female phase is figured and compared to the expected number (stippled fill) under the hypothesis of a 50:50 distribution. (A) small MP vs. large MP; (B) large MP vs. MFP; (C) small MP vs. MFP. $n = 30$ replicates in each treatment.

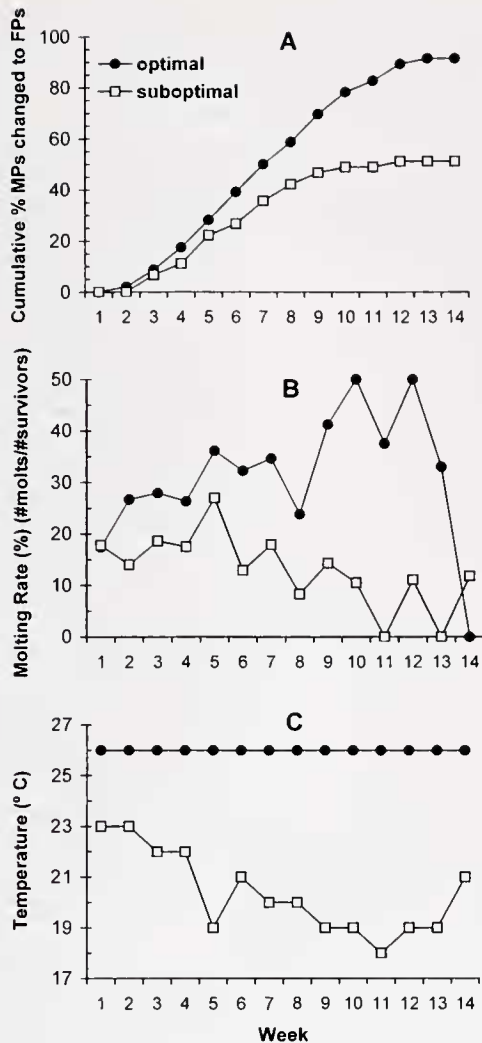


Figure 4. Change of large MPs to FPs under optimal and suboptimal conditions (treatments) during 14 weeks. (A) Cumulative percentage of large MPs changed to FP; (B) relative molting rates; (C) water temperatures.

the molting frequency, the average individual in the optimal treatment molted every 3.2 weeks *versus* every 7.7 weeks in the suboptimal treatment. Thus, during the first 14 weeks of the experiment, an average individual underwent at least 4 molts in the optimal and 1.8 molts in the suboptimal treatment.

After 14 weeks, only 2 MPs remained in the optimal treatment, but there were still 17 MPs in the suboptimal treatment. There had been no change to FP in the suboptimal treatment for 3 weeks (Figs. 4A, 5). At this time, the ambient laboratory temperature of the suboptimal treatment began to rise (Fig. 4C). To simulate a switch from suboptimal (fall/winter) to optimal (spring/summer) breeding conditions, the temperature and photoperiod of the suboptimal treatment was changed to equal that of the optimal. The experiment was followed for another 8 weeks. At the end of

this time, the two MPs of the original optimal treatment had not changed sex (verified by examination of pleopods after preservation). In the suboptimal treatment, after 6 weeks without any sex change and 3 weeks after the switch to optimal conditions, MPs began to change and continued to do so until the experiment was terminated (Fig. 5). In the last 8 weeks, 10 of 17 had changed to FP, while 2 others were late-stage transitionals (close to FP change) when removed from the experiment. Three of the original MPs in the suboptimal experiment remained, including one mid-stage transitional. Two others died during the last 8 weeks of the experiment.

At the beginning of the experiment, the mean size of MPs in the suboptimal ($n = 45$) and optimal ($n = 46$) treatments was equal (7.5 mm CL; t test, $P = 0.80$) (Fig. 6). The size of FPs (sex-changed individuals) was compared between the suboptimal (9.2 mm CL; $n = 33$) and optimal treatments (9.1 mm CL; $n = 40$) (Fig. 6), and the hypothesis of no difference is accepted (t test, $P = 0.58$). The size of FPs from the suboptimal treatment that transformed in weeks 14–22 (mean = 9.4 mm CL, $n = 10$) was slightly larger than that of FPs from the optimal treatment (Fig. 6), but the means of these two groups are not significantly different (t test, $P = 0.14$).

Discussion

This study addressed components of different hypotheses about the timing and degree of sex change in *Lysmata wurdemanni* that might explain the presence of large MPs (male-phase individuals) in this species. *Lysmata* spp. have a unique protandric sexual system in which sex-changed "females" (FPs) retain the ability to mate as males—that is, are simultaneous hermaphrodites (Bauer, 2000). The size (age) of change from MP to FP might be socially determined, resulting in considerable variation in sex change in

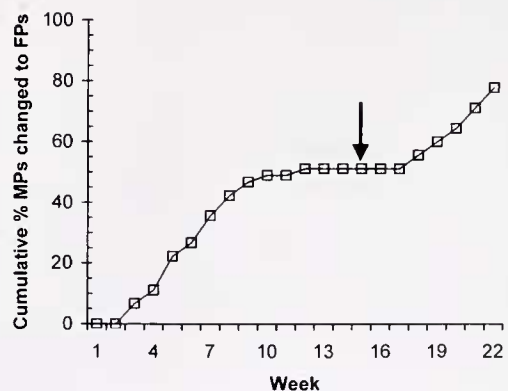


Figure 5. Change of large MPs to FPs in the suboptimal treatment during 22 weeks, first under suboptimal temperature and photoperiod for 14 weeks (as in Fig. 4A) and then after a change to optimal conditions (arrow) for the next 8 weeks.

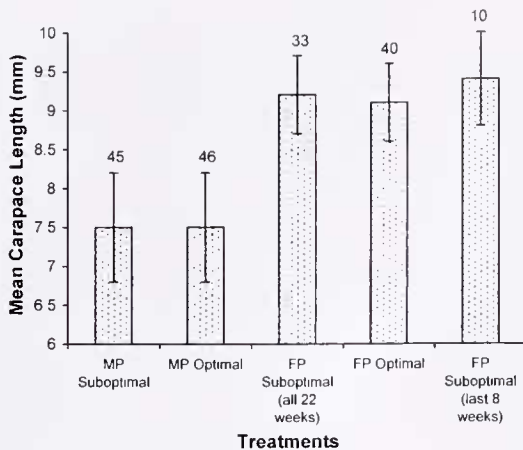


Figure 6. Sizes of MPs beginning experiment and of sex-changed individuals (FPs) in the large MP to FP sex change experiment. The number of individuals is given above each group. Error bars represent one standard deviation.

different demographic environments, as hypothesized for protandric pandalid shrimps (termed environmental sex determination, or ESD; Charnov *et al.*, 1978; Charnov, 1981; Charnov and Anderson, 1989). In ESD, the frequency of sexual morphs in the population (demographic environment), presumably perceived by contacts among individuals (socially mediated), influences the size and age at which males change to females. Delayed sex change might be advantageous to males in populations in which females are abundant. A sexually successful male might inseminate several females during the same period in which a female can produce only one brood of eggs, although those eggs are assured of fertilization by males.

However, "females" (FPs) of *L. wurdemanni* are simultaneous hermaphrodites that can mate as males (Bauer and Holt, 1998). The delayed sex change of MPs would only be adaptive, under an ESD hypothesis, if large MPs had a male mating advantage over the hermaphroditic FPs (Bauer and Holt, 1998; Bauer, 2000). In this study, I tested the relative male mating success of MPs and FPs by presenting a parturial FP simultaneously to a pair of potential male mating partners. Surprisingly, MFPs (FPs mating as males) were just as competitive as MPs, both large and small, in obtaining copulations from parturial FPs. Although these experiments demonstrated no male mating advantage of large MPs over FPs, large MPs were more successful than small MPs in obtaining the copulation of parturial FPs in a competitive situation. If FPs were not simultaneous hermaphrodites and thus could not mate as males, the superiority of large MPs over small MPs in inseminating parturial FPs would agree with an ESD explanation of delayed MP change to FP. However, there is no obvious benefit (selection pressure) favoring delayed change of a MP to a FP (a) whose male mating ability is equal to that of MPs, large and

small, and (b) whose reproduction as a female is not only assured but, given spawning every 2 weeks, would be more productive if change to FP occurred as early in life as morphologically and physiologically possible. In a population with few FPs, large MPs would have the mating advantage over small MPs, but there would be few FPs to exercise that advantage with. In a population with abundant FPs, large MPs would have more parturial FPs to mate with, but also more FPs, mating as males, to compete with for the same parturial FPs. Not surprisingly, the percentage of large MPs in the population was not significantly correlated with either low or high percentage of FPs. These results are not concordant with a hypothesis of socially determined sex change in *L. wurdemanni*. However, only large-scale experiments in which sex change in different demographic environments is measured can give a definite answer about whether or not socially mediated ESD occurs in *L. wurdemanni* or other *Lysmata* spp. Based on such large-scale experiments and parentage studies, Marliave *et al.* (1993) rejected the hypothesis of socially determined sex change in the protandric hermaphroditic shrimp *Pandalus danae*.

The hypothesis that FPs may change back to MPs in *Lysmata* under certain conditions, accounting for large MPs, was suggested by the anecdotal observations of colleagues rearing *L. wurdemanni* and by work on *Athanas indicus* (Suzuki, 1970) in which reversion of protandric FPs to MP was reported. Control of sexual differentiation by the androgenic glands (AG), located on the distal vas deferens (ejaculatory duct), has been demonstrated in various malacostracan crustaceans (Charniaux-Cotton and Payen, 1985; Hasegawa *et al.*, 1993). Both males and females have primordia of the AG, but its development in genetic males causes growth of male external characters and testes. However, it is still not established that AG hormone is the only factor controlling sex differentiation in Crustacea (Sagi *et al.*, 1997). Study of AGs in caridean shrimps is limited. Disappearance of the AGs after change to the female phase was reported, on the basis of stereomicroscopical and histological observations, in *L. seticaudata* (Berreur-Bonnenfant and Charniaux-Cotton, 1965). In *L. amboinensis*, Fiedler (1998) reported an absence of AGs in FPs, but neither similar histological examination nor other observations on MP androgenic glands was given for comparison. However, as in *L. wurdemanni*, male ducts with sperm production are retained in FPs of both species. Fiedler (1998) showed that FPs of *L. amboinensis*, like those of *L. wurdemanni*, mate as males and inseminate prespawning FPs. A small number of *L. wurdemanni* FPs show external male characters (reduced appendices masculinae) (Bauer and Holt, 1998). Obviously, male function is maintained. Hormones maintaining male characters in FPs might still be produced by remnants of AGs retained from the MP stage but overlooked in histological studies. There must be a balance of male and female hormones in the FPs that allows simultaneous male and

female function. Therefore, the possibility of change from FPs back to large MPs was tested in this study.

In the FP to MP sex change experiment, one group of FPs was maintained under optimal breeding conditions of high water temperature and long day length (Bauer and Holt, 1998; Lin and Zhang, 2001) approximating those found in late spring and summer at the collecting site (Bauer, 2002). Given the hypothesis of socially mediated ESD proposed for pandalid shrimps (Charnov, 1981), reversion to MP, if physiologically possible, might be expected in an all-FP group under optimal breeding conditions. However, mating experiments in this study showed no strong selective advantage for large MPs in a population with abundant FPs, and no reversion to the male phase was observed in the treatment with optimal breeding conditions.

The suboptimal treatment tested the possibility that FPs exposed to less favorable breeding conditions, such as lower water temperatures and short day length, might revert to large MPs. In many caridean species, females molt out of "breeding dress" (characters related to spawning and incubation) outside of the breeding season. In the simultaneous hermaphrodite FP of *L. wurdemanni*, it was hypothesized that suboptimal breeding conditions might tip the balance of male/female hormones away from the female phase back to the male phase. The FPs in this treatment were exposed to water temperatures and a photoperiod approximating late fall through early winter conditions in the natural habitat of the population sampled (Bauer, 2002). The less favorable breeding conditions of the suboptimal treatment are apparent from a comparison of the reproductive condition and molting rates between the suboptimal and optimal treatments. The percentage of FPs brooding embryos or preparing for spawning was only slightly higher in the optimal than in the suboptimal treatment. However, the molting rate of the optimal treatment, adjusted for number of FPs surviving, was more than twice that of the suboptimal treatment. In *L. wurdemanni* FPs, spawning occurs at every molt (Bauer and Holt, 1998), so that the higher molting of the optimal treatment indicates that more broods were produced. At the temperature and photoperiod of the optimal treatment, molting and spawning may occur every 10–14 days (Lin and Zhang, 2001; Bauer, pers. obs.). Using a figure of 12 days between spawning molts, a FP in the optimal treatment could have molted and spawned five times during the 9 weeks of the experiment. Molting in the suboptimal treatment was less than half of that in the optimal treatment, so that the number of FP molts in this treatment was about two, with a maximum of two spawns possible. Although breeding conditions were poorer in the suboptimal treatment, no reversion of FPs to MPs occurred. Given that change from MP to FP takes place within a single molt cycle, reversion of FPs back to a male phase could have taken place within a single molt cycle as well. In the suboptimal treatment, FPs passed through two molt

cycles, sufficient opportunity for reversion to the male phase, which did not, however, occur.

The high relative abundance of large MPs from fall through early spring might be best explained by suppression of sex change to FPs because of suboptimal breeding conditions. In the fall, MPs that have grown to and beyond the minimum size of sex change may not transform as they enter a period of the year not favorable for embryo production and larval survival. The proximate factors that retard sex change may be seasonally related environmental conditions such as lower temperature or shorter day length. The change of large MPs to FPs was much higher in conditions simulating spring/summer than in those simulating fall/winter conditions. Nearly all large MPs changed to FPs in the optimal treatment during the first 14 weeks of observation. There was much less change to FP during the same time period (14 weeks) when MPs were maintained under suboptimal (fall/winter) conditions. Molting rates were lower, but MPs of sufficient size to change to FP require only one molt cycle to do so (Bauer and Holt, 1998). The average MP in this treatment molted twice during the first 14 weeks.

After 14 weeks, the conditions of the suboptimal treatment were changed to those of the optimal treatment to determine if the slower rate of sex change could be increased in the surviving MPs. After 3 weeks (6 weeks without any MP to FP change), MPs began again to change to FP, and a majority did change or were changing to FP by the end of the experiment. During the same period, neither of the two MPs remaining in the original optimal treatment changed. At the end of 22 weeks two MPs from the original suboptimal treatment had neither changed nor showed signs of change to FP in spite of a switch from suboptimal to optimal conditions during the last 8 weeks of observation. Lin and Zhang (2001) found that all MPs reared individually under optimal conditions became FPs; but when MPs were reared in groups of 10, as in this study, only 67% transformed to FP, much less than that observed in the present study. Lin and Zhang began with postlarvae (MPs), and the total period of observation was not specified. In the present study, the experiment began with MPs at or above the minimum size of change to FP. Although Lin and Zhang suggested that a rather high percentage of individuals never change sex (primary males), my results suggest that most, if not all, will change to FP sometime during their lifetime if they live out the maximum natural lifespan (about 1.5 years; A. W. Baldwin and R. T. Bauer, unpubl.). However, in both experimental groups in my study, a low percentage of individuals (4%) never changed under laboratory conditions.

The adaptive advantage of not changing to FP during the non-breeding season may be that reproductive effort will be wasted on embryo production during a period unfavorable to larvae. This energy might be channeled into maintenance

and growth so that when spring arrives, the MP will change into a larger FP, with the benefit of a larger brood produced under conditions favorable to larval growth and survival. Brood size in *L. wurdemanni* is highly correlated with body size (Bauer, unpubl.), as in other carideans (Bauer, 1991). Suboptimal conditions did slow and then stop sex change in this study, explaining the presence of large and very large MPs during the fall through early spring in field samples. However, large MPs did not change to FP at a significantly larger size than large MPs reared under optimal (spring/summer) conditions.

In conclusion, mating experiments showed that FPs are competitive with MPs of any size in obtaining copulations with parturial FPs. There seems to be no mating advantage for an individual to remain a large MP, and under optimal breeding conditions, almost all change to FP. The proportion of large and very large MPs in the natural population studied is greatest from fall through early spring, and in the laboratory, change from MP to FP is slowed under the suboptimal breeding conditions of these seasons. Seasonal changes in abiotic proximate factors that regulate breeding, such as temperature and photoperiod, seem a more likely explanation for the occurrence of large MPs in *Lysmata wurdemanni* than does socially mediated ESD in different demographic situations. In *L. wurdemanni*, there may also be genetic variation in the size of sex change, although adaptive advantages to change at a larger size are not apparent from this and previous studies.

The proportion of FPs was often low in the *Lysmata wurdemanni* population sampled (Fig. 1). This is partly because all smaller (younger) individuals of this sex-changing species are male phase. In addition, as shown in this report and elsewhere (Bauer and Holt, 1998; Bauer, 2000), many large MPs capable of changing to FP do not do so for an extended period, so that the population often has a low proportion of FPs (Fig. 1). Therefore, the frequent occurrence of an extended male phase results in a lower number of FPs in the population (Fig. 1). This may affect the total output of larvae and subsequent recruitment potential of the population.

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